PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	
H 1987 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006617	18.06.2004	20.06.2003

International Patent Classification (IPC) or national classification and IPC
C12N15/10

Applicant
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1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 24 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.01.2005	01.08.2005

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/006617

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-101 as originally filed

   Claims, Numbers

   1-36 as originally filed

   Drawings, Sheets

   1/13-13/13 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 5-12, 22-29, 35-36 (partially) and 30-31 (completely)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 5-12, 22-29, 35-36 (partially) and 30-31 (completely)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-29, 32-36 (partially) .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12, 22, 25-29,32,34
	No:	Claims	13-21,23-24,35
Inventive step (IS)	Yes:	Claims	5, 25-29,32,34
	No:	Claims	1-4,6-12,22
Industrial applicability (IA)	Yes:	Claims	1-29, 32-36
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

ITEM III:

1. Present **claim 5** relates to the method of any one of claims 1-4, wherein said disease-related protein is huntingtin and wherein said interaction partners are the interaction partners as shown in Tab. 6, 7 or 9.

However, since Tab. 6 and Tab. 9 comprise proteins without any reference to an Accession Number, the said claim is so unclear that a meaningful search is rendered impossible over the whole scope of the claim.
Consequently, only those part of the claim which refer to the Tab. 7 for which Accession Numbers are provided for the different cited proteins have been taken into consideration for the assessment of non unity and, therefore, for possible searches.

The same applied to **claims 6-12**, insofar as dependent on claim 5.

2. Present **claim 22** relates to a protein complex comprising at least two proteins, wherein said at least two proteins are selected from the group of interaction partners listed in Tab. 9.

For the same reasons as set out under item 1 herein above, the said claim is so unclear that a meaningful search over the whole scope is not possible.
Consequently, only the protein complexes for which the Accession Number of proteins is disclosed in Tab. 7 have been considered when defining the inventions in the context of the non-unity objections.

The same applied to **claims 23-24**, insofar as dependent on claim 22.

3. Present **claims 25-28**, relates to a method of identifying whether a protein promotes (claim 25) or inhibits (claim 26) huntingtin aggregation wherein a modulator protein is selected from Tab. 6 or 7.

For the same reasons as set out under item 1 herein above, the said claims are so unclear that a meaningful search is rendered impossible over the whole scope of the claim.
Consequently, the inventions have been defined based on those part of the claims which relate to Tab. 7 for which accession numbers are provided for the different cited proteins.

4. The same applies to the present **claim 29**, which relates to a method for

identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin and to **claims 35-36**.

5. **Claims 30-31** do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claim attempts to define the subject-matter only in terms of a functional feature of the compound, namely that it has been identified by the method of claims 25-29. However, since this feature does not provide any indication as to the structure of the said compound and since **claims 30-31** are silent as to the compound which is modelled, synthesized (claim 30) and further modified (claim 31), the said claims lack clarity to such an extend as to render a meaningful search impossible. Moreover, the description does not disclose any such compound either.

Thus, said **claims 30-31** cannot been searched (see PCT/ISA form 206) and have not been taken into account for the assessment of non-unity. No opinion will thus be given with respect to novelty, inventive step or industrial applicability.

ITEM IV:

The separate inventions are:

Invention 1: claims 1-29, 32 and 35-36 (all partially).

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, and in particular when the disease-related protein is huntingtin and more particularly when the modulator of huntingtin is BARD1.
A nucleic acid molecule encoding a modulator of huntingtin wherein said modulator is BARD1.
A vector and a host cell comprising a nucleic acid molecule encoding BARD1.
A polypeptide comprising an amino acid sequence of BARD1.
A method of producing the polypeptide comprising an amino acid sequence of BARD1.
A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second one is SETDB1.
An antibody specifically recognising BARD1.
A method of identifying whether a protein promotes or inhibits huntingtin

aggregation wherein the protein is BARD1.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is BARD1.
A method of diagnosing Huntington's disease in a biological sample using BARD1.
A diagnostic agent/composition or pharmaceutical composition using BARD1.

Invention 1.1: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is CA150.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.2: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is NAG4.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.3: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is HIP15.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.4: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is PTN.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.5: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is FEZ1.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.6: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is IKAP.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.7: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is BAIP1.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.8: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is mHAP1.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.9: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is HBO1.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.10: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is BAIP2.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.11: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is PLIP.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.12: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is PIASy.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.13: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is HZFH.
An antibody specifically recognizing the protein complex as set herein above.

Invention 1.14: claims 22-24 (partially).

A protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is ZHX1.
An antibody specifically recognizing the protein complex as set herein above.

Inventions 2-5: claims 5-29 (partially), 32 (partially), 35-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, wherein the disease-related protein is huntingtin and the modulator of huntingtin is one of the modulators of Tab. 8, different from BARD1 and GIT1.
A nucleic acid molecule encoding a modulator of huntingtin wherein said modulator is one of the modulators of Tab. 8, different from BARD1 and GIT1.
A vector and a host cell comprising the nucleic acid molecule as set out herein above.
A polypeptide comprising an amino acid sequence encoding one of the modulators as set out herein above.
A method of producing the polypeptide as set out herein above.
A protein complex comprising at least two proteins, wherein the first protein is one of the other modulators as set out herein above.
An antibody specifically recognising one of the other modulators as set out herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is one of the proteins of Tab. 8, different from BARD1 and GIT1.
A method for identifying compounds affecting an interaction of huntingtin or of a

direct or indirect interaction partner of huntingtin wherein said the compound is one of the compounds of Tab. 8, different from BARD1 and GIT1.
A method of diagnosing Huntington's disease in a biological sample using one of the compounds of Tab. 8, different from BARD1 and GIT1.
A diagnostic agent/composition or pharmaceutical composition using one of the compounds of Tab. 8, different from BARD1 and GIT1.

Invention 6: claims 5-29 (partially), 32 (partially), 33 (completely), 35-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, wherein the disease-related protein is huntingtin and the modulator of huntingtin is GIT1.
A nucleic acid molecule encoding a modulator of huntingtin wherein said modulator is GIT1.
A vector and a host cell comprising the nucleic acid molecule as set out herein above.
A polypeptide comprising an amino acid sequence encoding GIT1.
A method of producing the polypeptide as set out herein above.
A protein complex comprising at least two proteins, wherein the first protein is GIT1.
An antibody specifically recognising GIT1 or the complex as defined herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is GIT1.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is GIT1.
A method of diagnosing Huntington's disease in a biological sample using GIT.
A diagnostic agent/composition or pharmaceutical composition using GIT1.

Invention 7: claims 5-12 (partially), 22-29 (partially), 32 (partially), 34-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, wherein the disease-related protein is huntingtin and the modulator of huntingtin is htt as defined in Tab. 7.
A method of producing the polypeptide comprising an amino acid sequence

encoding htt.
A protein complex comprising at least two proteins, wherein the first protein is htt as defined in Tab. 7.
An antibody specifically recognising htt or the complex as defined herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is htt.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is htt.
A method of diagnosing Huntington's disease in a biological sample using htt.
A diagnostic agent/composition or pharmaceutical composition using htt.

Invention 8: claims 5-12 (partially), 22-29 (partially), 32 (partially), 34-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, wherein the disease-related protein is huntingtin and the modulator of huntingtin is HIP15.
A protein complex comprising at least two proteins, wherein the first protein is HIP15.
An antibody specifically recognising HIP15 or the complex as defined herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is HIP15.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is HIP15.
A method of diagnosing Huntington's disease in a biological sample using HIP15.
A diagnostic agent/composition or pharmaceutical composition using HIP15.

Invention 9: claims 5-12 (partially), 22-29 (partially), 32 (partially), 34-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide, wherein the disease-related protein is huntingtin and the modulator of huntingtin is HP28.
A protein complex comprising at least two proteins, wherein the first protein is HP28.

An antibody specifically recognising HP28 or the complex as defined herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is HP28.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is HP28.
A method of diagnosing Huntington's disease in a biological sample using HP28.
A diagnostic agent/composition or pharmaceutical composition using HP28.

Inventions 10-73: claims 5-12 (partially), 22-29 and 32 (partially), 35-36 (partially)

A method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide,, wherein the disease-related protein is huntingtin and the modulator of huntingtin is a protein as listed in Tab. 7, insofar as the said protein does not relate to those listed in Tab. 8, to BARD1, htt, HIP15 and HP28. Moreover, for those proteins present both in Tab. 7 and in Tab. 9, the inventions will also comprise a protein complex comprising at least two proteins, wherein the first protein is one of the protein disclosed in Tab. 9, insofar as the said protein does not relate to those listed in Tab. 8, to BARD1, htt, HIP15 and HP28.
An antibody specifically recognising the complex as defined herein above.
A method of identifying whether a protein promotes or inhibits huntingtin aggregation wherein the protein is one of the proteins selected in the Tab. 7 but different from BARD1, from those listed in Tab. 8 and from htt, HIP15 and HP28.
A method for identifying compounds affecting an interaction of huntingtin or of a direct or indirect interaction partner of huntingtin wherein said the compound is one of the proteins selected in the Tab. 7 but different from BARD1, from those listed in Tab. 8 and from htt, HIP15 and HP28.
A method of diagnosing Huntington's disease in a biological sample using one of the proteins selected in the Tab. 7 but different from BARD1, from those listed in Tab. 8 and from htt, HIP15 and HP28.
A diagnostic agent/composition or pharmaceutical composition using one of the proteins selected in the Tab. 7 but different from BARD1, from those listed in Tab. 8 and from htt, HIP15 and HP28.

1- Unity of invention can only be acknowledged if the application relates to a group of

inventions linked by a single general inventive concept, i.e. when all inventions involve the same or corresponding special technical features (R. 13.1 and 13.2 PCT). Moreover, in the case wherein the claims relate to a plurality of alternative chemical compounds, the PCT Guidelines (see 10.17) require that unity can only be acknowledged if all the compounds have a common property and share a significant structural element.

A common concept that could link, as required by R. 13 PCT, the different methods to which independent claims 1, 25, 26, 29 and 32 relate could be seen in a method for identifying a network of direct and indirect interaction partners of a disease-related (poly)peptide, whereby only by such a method it was possible to identify molecules suitable for the methods to which claims 25, 26, 29 and 32 relate.

However, a method for identifying a network of direct and indirect interaction partners of a disease-related (poly)peptide is known from D1 (Zanzoni A. et al., 2002).
D1 relates to a molecular interaction database called MINT wherein:

i) both direct and indirect interactions are considered (abstract),
ii) the p53 tumor suppressor protein interaction network is disclosed (Fig. 2)
iii) any entity can interact with any other entity in the database by binding (p. 136, c. 1, § 2),
iv) detection of protein-protein interactions are performed by different methods including two-hybrid, co-immunoprecipitation (Fig. 3).

D2 (Sittler A. et al. October 1998) relates to SH3GL3 as a new huntingtin-interacting protein (see abstract), wherein SH3GL3 is one of the example of modulator of huntingtin cited in the present application (see p. 18, l. 9; Tab. 7).

D3 (WO-03/045990) relates to protein-protein interaction wherein HYPA is cited as a known huntingtin-interacting protein (see example 14, p. 56-59) and wherein HYPA is one of the example of modulator of huntingtin cited in the present application (see Tab. 7).

D4 (US-6235879) relates to a family of apoptosis modulators that interact with the Huntington's Disease (HD) gene product wherein:

i) co-transfection of HIP-1 and HD1955 was used to test the influence of HIP-1 on huntingtin aggregation in comparison to a control expressing HD1955 (c. 19, l. 30-45),
ii) in the control transfections, 1-2% of cells expressing HD1955-128 formed aggregates in the absence of tamoxifen, similar to HD1955-128 expressed alone (c. 19, l. 30-45),
iii) when HD1955-128 was co-expressed with HIP-1, an average of 14% of huntingtin-expressing cells contained aggregates with no tamoxifen treatment, directly implicating HIP-1 in the increase in aggregation (c. 19, l. 30-45).
iv) probes encoding portions of HIP-1 can also be used for diagnostic purposes to characterize risk of Huntington's Disease (c. 4, l. 28-32).

Since D1 discloses a direct and indirect interaction proteins network and cites as example the p53 tumor suppressor protein interaction network, and since modulators of huntingtin, as well as proteins modulating huntingtin aggregation or to be used for the diagnosis of HD are also known from D2, D3 or D4, the aforementioned concept cannot be regarded as the single general inventive concept linking the said inventions or groups of inventions.

The only further common concept linking the different claims could be identified in the specific molecules to which claims 5 (which depends on claim 1), 25, 26, 29 and 32 relate, i.e. those of table 7.
The application contends that all these molecules play a role in HD: the first criteria set out in the PCT Guidelines appears thus to be met. However, contrary to the requirements set out in the Guidelines, these molecules do not share any significant structural element. Therefore, each of them relates to a different invention.

In view of the foregoing, the present claims relate to inventions which are not linked by a single general inventive concept and relate thus to 73 separate inventions listed hereinabove.

2- Furthermore, the specific method to which claim 1 relates lacks an inventive step over Zanzoni et al. (D1).

D1 relates to a molecular interaction database called MINT wherein:

i) both direct and indirect interactions are considered (abstract),
ii) the p53 tumor suppressor protein interaction network is disclosed (Fig. 2)
iii) any entity can interact with any other entity in the database by binding (p. 136, c. 1, §. 2),
iv) detection of protein-protein interactions are performed by different methods including two-hybrid, co-immunoprecipitation (Fig. 3).

The subject-matter of claim 1 differs from that of D1, which is considered as the closest prior art, in that the steps of the method are different.

In view of the absence of any technical feature characterising the different steps of the method disclosed in claim 1 and since the application is silent as to any surprising effect linked to the method, the technical problem to be solved by the subject-matter of said claim 1 may be regarded as providing an alternative method to that of D1.

Since D1 discloses a method to obtain a direct and indirect interaction proteins network and cites as example the p53 tumor suppressor protein interaction network and in view of the absence of any technical features characterising the different steps of the method disclosed in claim 1 and since the application is silent as to any surprising effect linked to the method, the method of claim 1 appears to relate to an arbitrary succession of steps.

The subject-matter of claim 1 does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

In view of this, unity between the method and any product obtainable thereby is absent. However, in view of the non excessive amount of work required, the ISA decided to search both the general invention to which claims 1-4 relate as well as all claimed products and methods based on the first gene mentioned in table 7, namely BARD1.

Item V:

6. INTRODUCTION

1. The following documents (D1-D6) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: Zanzoni A. et al. (2002), MINT: a Molecular INTeraction database. FEBS

Letters 513 : 135-140.

D2: Sittler A. et al., (October 1998), SH3GL3 associates with the huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. Molecular Cell 2 : 427-436.

D3: WO-A-03/045990.

D4: US-A-6235879

D5: Kleinman F.E. and Manley J.L. (3 September 1999), Functional Interaction of BRAC1-associated BARD1 with polyadenylation factor CstF-50. Science 285 : 1576-1579.

D6: Thai T.H. et al. (1998), Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. Human Molecular Genetics 7(2) : 195-202.

2. The present application relates to a method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide.

7. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT)

1. No cited prior-art document discloses a method for generating a network of indirect and indirect interaction partners of a disease-related (poly)peptide as disclosed in **claim 1**.

Therefore, the subject-matter of independent **claim 1** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of dependent **claims 2-12** is also considered novel in the sense of Art. 33(2) PCT.

However, the subject-matter of **claim 1** does not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).
D1, which is considered as the closest prior art, relates to a molecular

interaction database called MINT wherein:

I) both direct and indirect interactions are considered (abstract),

ii) the p53 tumor suppressor protein interaction network is disclosed (Fig. 2)

iii) any entity can interact with any other entity in the database by binding (p. 136, c. 1, §. 2),

iv) detection of protein-protein interactions are performed by different methods including two-hybrid, co-immunoprecipitation (Fig. 3).

The subject-matter of **claim 1** differs from that of D1, which is considered as the closest prior art, in that the steps of the method are different.

In view of the absence of any technical feature characterising the different steps of the method disclosed in **claim 1** and since the application is silent as to any surprising effect linked to the method, the technical problem to be solved by the subject-matter of said **claim 1** may be regarded as providing an alternative method to that of D1.

Since D1 discloses a method to obtain a direct and indirect interaction proteins network and cites as example the p53 tumor suppressor protein interaction network and in view of the absence of any technical features characterising the different steps of the method disclosed in **claim 1** and since the application is silent as to any surprising effect linked to the method, the method of **claim 1** appears to relate to an arbitrary succession of steps.

The subject-matter of **claim 1** does, therefore, not involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

Moreover, dependent **claims 2-4** do not appear to add anything inventive because they relate to common place features (Art. 33(3) PCT).

However, the subject-matter of **claim 5,** which relates to the method of claim 1 wherein the disease-related protein is huntingtin and the interaction partners is BARD1, involves an inventive step (Art. 33(3) PCT).

The subject-matter of **claim 5** differs from that of D2, which is considered as the closest prior art, in that BARD1 is identified as an interaction partner of huntingtin.

Thus, the technical problem to be solved by the subject-matter of said **claim 5** may be regarded as providing a alternative interaction partners to huntingtin to that of D2, namely SH3GL3.

In view of the absence of any indications which would have lead the skilled

person to study the interaction between huntingtin and BARD1, it would not have been obvious for the skilled person to develop a method for generating a network of direct and indirect partners wherein the disease-related protein is huntingtin and the interaction partners is BARD1 such as the one claimed in the present application.
Therefore, the subject-matter of **claim 5** involves an inventive step (Art. 33(3) PCT).

Claim 6 does not involve an inventive step in view of D1 (Art. 33(3) PCT) for the same reasons as set out under the items herein above and because it is common practice in molecular biology to determine the nucleic acid sequence of a protein.

Claim 7 does not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.
The subject-matter of **claim 7** differs from that of D1, which is considered as the closest prior art, in that the proteins are translated from a nucleic acid library.
D3 relates to protein-protein interactions involved in transforming growth factor beta disorders and/or diseases wherein:

I) example 2 provides a method of screening with the two-hybrid in yeast system (p. 34-37),

ii) the prey polynucleotide that has been selected by testing the library of preys in a screen using the two-hybrid, two plus one hybrid methods and the like, encodes the polypeptide interacting with the protein of interest (p. 22, l. 12-16).

In absence of any surprising effect linked to the use of proteins translated from a nucleic acid library and since D3 teaches that polypeptides interacting with the protein of interest are encoded by prey polynucleotide that has been selected by testing the library of preys, it would be then obvious for the skilled person to combine the method of D1 with the teaching of D3, thereby arriving at the method of **claim 7**.
The subject-matter of **claim 7** does, therefore, not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.

The subject-matter of **claims 8 and 9** does not involve an inventive step in view of D1 in the sense of Art. 33(3) PCT because the protein interaction database

of D1 stores protein interaction information disseminated in the scientific literature which means that the proteins forming the network of D1 have different sources.

Claim 10 does not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.
The subject-matter of **claim 10** differs from that of D1, which is considered as the closest prior art, in that proteins are contacted on an array.
Since protein microarrays are well known in the art (see the method disclosed in D3 which relates to protein chips or protein microarrays wherein proteins attach covalently to the slide surface retain their ability to interact with other proteins or small molecules in solution (p. 31, l. 27-31)), it would be then obvious for the skilled person to combine the teaching of D3 with the method of D1, thereby arriving at the method of **claim 10**.
The subject-matter of **claim 10** does, therefore, not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.

The same applied to **claim 11**, which relates to the well known technique of Y2H (see D3 that provides an example of yeast two-hybrid (see example 2, p. 34-37)). It would be then obvious for the skilled person to use the Y2H in the method of D1, thereby arriving at the method of **claim 11**.
The subject-matter of **claim 11** does, therefore, not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.

D3 disclosed examples of huntingtin partners with their nucleic acid sequences (see example 14, p. 57-59). Since it is common practice in the art to deduce the nucleic acid sequence from a protein, it would be obvious for the skilled person to combine the teaching of D3 with the method of D1, thereby arriving at the method of **claim 12**.
The subject-matter of **claim 12** does, therefore, not involve an inventive step in view of D1 combined with D3 in the sense of Art. 33(3) PCT.

2. The present application does not meet the requirements of Art. 33(2) PCT, because the subject-matter of **claims 13-21, 23-24 and 35** is not new over D5.

D5 relates to the functional interaction of BARD1 with polyadenylation factor

CstF-50 wherein:

I) to identify additional CstF-50-interacting proteins, two hybrid assays are performed (p. 1576, c. 2, §. 2),

ii) a cDNAs library from human feotal brain RNA is screened (p. 1578, c. 3, §. 2),

iii) BARD1 is recovered among the strongest interacters (p. 1576, c. 2, §. 2),

iv) the BARD1 cDNAs is obtained from the two-hybrid screen (p. 1576, c. 2, §. 3),

v) GST-BARD1 derivatives are used (Fig. 1);

vi) for GST fusion protein interaction assays, cDNAs encoding full-length or truncated BARD1 are inserted into pGEX-2TK and expressed in E.coli (p. 1579, c. 1, §. 1),

vii) an anti-BARD1 monoclonal antibody is used (p. 1577, c. 1, §. 3).

3. However, no cited prior-art document discloses a protein complex comprising at least two proteins, wherein the first protein is BARD1 and the second is SETDB1 or CA150, NAG4, HIP15, HIP5, PTN, FEZ1, IKAP, BAIP1, mHAP1, HBO1, BAIP2, PLIP, PIASy, HZFH and ZHX1, as disclosed in **claim 22.**

Therefore, the subject-matter of independent **claim 22** is considered novel in the sense of Art. 33(2) PCT.

However, the subject-matter of **claim 22** does not involve an inventive step in the sense of Art. 33(3) PCT.

D5 shows the formation of a complex between BARD1 and CstF-50 which is immunoprecipited with anti-BARD1 antibodies (Fig. 2).

In absence of any particular effect linked to the BARD1-SETDB1 complex and since high-throughput methods for the identification of proteins interacting with disease-related protein encompass the study of protein-protein complex formation, the choice of the BARD1-SETDB1 complex appears as an arbitrary choice which does not involve any inventive step in the sense of Art. 33(3) PCT.

The same applied to the other complexes of Tab. 9 comprising BARD1.

4. No cited prior-art document discloses a method for identifying wether a protein promotes or inhibits huntingtin aggregation and wherein the

protein is BARD1, as disclosed in **claims 25-26.**

Therefore, the subject-matter of independent **claims 25-26** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of **claims 25-26** does involve an inventive step over the disclosure of D4 (Art. 33(3) PCT).

D4, which is considered as the closest prior art, relates to a family of apoptosis modulators that interact with the Huntington's Disease gene product wherein:

I) co-transfection of HIP-1 and HD1955 was used to test the influence of HIP-1 on huntingtin aggregation in comparison to a control expressing HD1955 (c. 19, §. 3),

ii) in the control transfections, 1-2% of cells expressing HD1955-128 formed aggregates in the absence of tamoxifen, similar to HD1955-128 expressed alone (c. 19, §. 3),

iii) when HD1955-128 was co-expressed with HIP-1, an average of 14% of huntingtin-expressing cells contained aggregates with no tamoxifen treatment, directly implicating HIP-1 in the increase in aggregation (c. 19, §. 3).

The subject-matter of **claims 25-26** differs from that of document D4 in that BARD1 is used as modulator of huntingtin aggregation.

Thus, the technical problem to be solved by the subject-matter of said **claims 25-26** may be regarded as providing a method employing an alternative modulator to that of D4.

In view of the absence of any indications which would have lead the skilled person to use BARD1 as modulator of huntingtin aggregation in the method of D4, it would not have been obvious for the skilled person to develop a method of identifying wether BARD1 promotes or inhibits huntingtin aggregation.

Therefore, the subject-matter of **claims 25-26** involves an inventive step (Art. 33(3) PCT).

Dependent **claims 27-29** further define specific embodiments of the novel and inventive method of claims 25-26.

Dependent **claims 27-29** are hence also considered to meet the requirements of (Art. 33 (1), (2) and (3) PCT).

5. No cited prior-art document discloses a method of diagnosing Huntington's disease in a biological sample using anti-BARD1

antibodies, as disclosed in **claim 32.**

Therefore, the subject-matter of independent **claim 32** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of **claim 32** does involve an inventive step over the disclosure of D4 (Art. 33(3) PCT).

D4, which is considered as the closest prior art, relates to a family of apoptosis modulators that interact with the Huntington's Disease gene product wherein probes encoding portions of HIP-1 can also be used for diagnostic purposes to characterize risk of Huntington's Disease (c. 4, §. 3).

The subject-matter of **claim 32** differs from that of document D4 in that anti-BARD1 antibodies are used for diagnosing Huntington's disease.

Thus, the technical problem to be solved by the subject-matter of said **claim 32** may be regarded as providing an alternative diagnosing method to that of D4.

In view of the absence of any indications which would have lead the skilled person to use anti-BARD1 antibodies for diagnosing Huntington's disease, it would not have been obvious for the skilled person to develop a method as disclosed in **claim 32.**

Therefore, the subject-matter of independent **claim 32** does involve an inventive step over the disclosure of D4 in the sense of Art. 33(3) PCT.

Dependent **claim 34** further defines specific embodiments of the novel and inventive method of claim 32.

Dependent **claim 34** is hence also considered to meet the requirements of (Art. 33 (1), (2) and (3) PCT).

6. The present application does not meet the requirements of Art. 33(2) PCT, because the subject-matter of **claim 35** is not new over D6.

D6 relates to mutations in BARD1 gene in primary breast, ovarian and uterine cancers wherein a germline alteration of BARD1, namely Gln561His, is diagnosed with primary cancers (see abstract).

The subject-matter of **claim 35** is, therefore, not new over D6 in the sense of Art. 33(2) PCT.

7. No cited prior-art document discloses the use of BARD1 for the preparation of a pharmaceutical composition for the treatment of Huntington's disease.

Therefore, the subject-matter of **claim 36** is considered novel in the sense of Art. 33(2) PCT.

However, since the present description is silent as to any therapeutic effect of BARD1 in the treatment of Huntington's disease, the subject-matter of independent **claim 36** does, therefore, not involve an inventive step in the sense of Art. 33(3) PCT.

ITEM VIII:

Present **claim 1** relates to a method for generating a network of direct and indirect interaction partners of a disease-related (poly)peptide. However, the expression "direct and indirect interaction partners" is vague and unclear since the term "partner" does not contain any technical or structural feature which would allow the skilled person to understand it. Moreover, the expression "direct and indirect interaction" is so vague that it is not possible for the skilled person to identify the nature of the mentioned interactions.